

Claims

1. A protein having luciferase activity and at least 60% similarity to luciferase from *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata*, *Luciola lateralis*, *Hotaria paroula*, *Pyrophorus plagiophthalmus*, *Lampyrus noctiluca*, *Pyrocoelia nayako* or *Photinus pennsylvanicus*, wherein in the sequence of the enzyme, at least one of
- (a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase or to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;
- (b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase or to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;
- (c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase or to residue 297 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;
- (d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to residue 16 of *Luciola mingrelica*, or 17 of *Luciola cruciata* or *Luciola lateralis*;
- (e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to residue 37 of *Luciola mingrelica*, or 38 of *Luciola cruciata* or *Luciola lateralis*;
- (f) the amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase or to residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene;
- (g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase or to residue 236 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;
- (h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase or to residue 422 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;
- (i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase or to residue 312 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;

is different to the amino acid which appears in the corresponding wild type sequence and wherein the luciferase enzyme has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.

2. A protein according to claim 1 which has the sequence of a wild-type luciferase, in which more than one amino acid residue is different to that of the wild type enzyme.

3. A protein according to claim 2 wherein up to 50 amino acids are different to that of the wild type enzyme.

a 4. A protein according to ^{Claim 1} ~~any one of the preceding claims~~ wherein the luciferase is a modified form of luciferase of *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase.

a 5. A protein according to ^{Claim 1} ~~any one of the preceding claims~~ wherein the sequence of luciferase of *Photinus pyralis*, wherein at least one of

(a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase is other than threonine;

(b) the amino acid residue corresponding to residue 232 in

Photinus pyralis luciferase is other than isoleucine;

(c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is other than phenylalanine;

(d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase is other than phenylalanine;

(e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase is other than leucine;

(f) amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase is other than alanine;

(g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase is other than aspartic acid;

(h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase is other than serine;

(i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase is other than histidine.

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6. A protein according to ^{Claim 1}~~any one of claims 1 to 4~~ wherein
- 5 protein has substantially the sequence of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* enzyme, and wherein at least one of
- (a) the amino acid residue corresponding to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*
- 10 luciferase is other than glycine (for *Luciola mingrelica* based sequences) or asparagine (for *Luciola cruciata* or *Luciola lateralis*) based sequences;
- (b) the amino acid residue corresponding to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*
- 15 luciferase is other than serine;
- (c) amino acid residue corresponding to residue 297 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than leucine;
- (d) amino acid residue corresponding to amino acid 16 of
- 20 *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than phenylalanine;
- (e) amino acid residue corresponding to residue 37 of *Luciola mingrelica*, or residue 38 of *Luciola cruciata* and *Luciola lateralis* is other than lysine;
- 25 (f) amino acid residue corresponding to amino acid residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis*, or 108 of *Luciola lateralis* gene is other than glycine;
- (g) amino acid residue corresponding to amino acid residue 236
- 30 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than glycine;
- (h) amino acid residue corresponding to residue 422 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than threonine;
- 35 (i) amino acid residue corresponding to amino acid residue 312 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than threonine (for *Luciola mingrelica* based sequences)

or valine (for *Luciola cruciata* or *Luciola lateralis*) based sequences.

a 7. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} wherein comprising a protein having luciferase activity and at
5 least 60% similarity to luciferase from *Photinus pyralis*,
Luciola mingrelica, *Luciola cruciata* or *Luciola lateralis*
enzyme wherein in the sequence of the enzyme, at least one of
(a) the amino acid residue corresponding to residue 214 in
10 *Photinus pyralis* luciferase and to residue 216 of *Luciola*
mingrelica, *Luciola cruciata* or *Luciola lateralis* luciferase is
mutated and is other than threonine in the case of *Photinus*
pyralis luciferase; or
(b) the amino acid residue corresponding to residue 232 in
15 *Photinus pyralis* luciferase and to residue 234 of *Luciola*
mingrelica, *Luciola cruciata* or *Luciola lateralis* luciferase is
mutated and is other than isoleucine in the case of *Photinus*
pyralis luciferase; or
(c) amino acid residue corresponding to residue 295 in *Photinus*
20 *pyralis* luciferase and to residue 297 of *Luciola mingrelica*,
Luciola cruciata or *Luciola lateralis* luciferase is mutated and
is for example, other than phenylalanine in the case of
Photinus pyralis luciferase;
and the luciferase enzyme has increased thermostability as
25 compared to the wild-type luciferase.

8. A protein according to claim 1 wherein the amino acid
residue corresponding to residue 214 in *Photinus pyralis*
luciferase and to residue 216 of *Luciola mingrelica*, *Luciola*
30 *cruciata* or *Luciola lateralis* luciferase is alanine.

a 9. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} wherein the amino acid residue corresponding to residue 232 in
Photinus pyralis luciferase and to residue 234 of *Luciola*
35 *mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is
alanine.

a 10. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} which is a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is leucine.

5 a 11. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} wherein the amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to amino acid 16 in *Luciola* luciferase, is alanine.

10 c 12. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} wherein the luciferase is a mutated luciferase of *Photinus pyralis* or a *Luciola* species where the amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to amino acid residue 37 in *Luciola mingrelica* or 15 38 of *Luciola lateralis* or *cruciata* luciferase is alanine.

a 13. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} wherein the amino acid residue corresponding to residue 105 in 20 *Photinus pyralis* luciferase and to residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene luciferase is valine.

a 14. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} which comprises a mutated *Photinus pyralis* luciferase wherein 25 the amino acid residue corresponding to residue 234 in *Photinus pyralis* luciferase is glycine.

a 15. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} which comprises a mutated *Photinus pyralis* luciferase wherein 30 the amino acid residue corresponding to residue 420 in *Photinus pyralis* luciferase is threonine.

a 16. A protein according to ~~any one of the preceding claims~~ ^{Claim 1} which comprises a mutated *Photinus pyralis* luciferase wherein 35 the amino acid residue corresponding to residue 310 in *Photinus pyralis* luciferase is arginine.

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Claim 1

17. A protein according to ~~any one of the preceding claims~~ wherein the amino acid at position corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in *Luciola* luciferase) is other than glutamate.

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18. A protein according to claim 17 wherein the amino acid at position corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in *Luciola* luciferase) is lysine or arginine.

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Claim 1

19. A protein according to ~~any one of the preceding claims~~ wherein the amino acid at the position corresponding to amino acid 217 in *Luciola* luciferase (215 in *Photinus pyralis*) is a different hydrophobic amino acid.

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20. A protein according to claim 19 wherein the amino acid at the position corresponding to amino acid 217 in *Luciola* luciferase (215 in *Photinus pyralis*) is isoleucine, leucine or valine.

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Claim 1

21. A nucleic acid which encodes a luciferase according to ~~any one of the preceding claims~~.

22. A vector comprising a nucleic acid according to claim 21.

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23. A cell transformed with a vector according to claim 22.

24. A cell according to claim 23 which is a prokaryotic cell.

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25. A cell according to claim 23 which is a plant cell.

26. A plant comprising cells according to claim 25.

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27. A method of producing a protein according to any one of claims 1 to 20, which method comprises culture of a cell according to claim 23 or growth of a plant according to claim 26.

28. The use of a protein according to ~~any one of claims 1 to 20~~ ^{Cl. 11} in a bioluminescent assay.

29. A kit comprising a protein according to ^{any one of claims}
1 to 20.

30. A kit according to claim 29 which further comprises luciferin.